Factors Affecting Humic-Nickel Complex Mediated Reduction of Trichloroethene in Homogeneous Aqueous Solution

H U I Z H O N G   M A ,
E D W A R D   J .   O ' L O U G H L I N ,   A N D
D A V I D   R .   B U R R I S *

Air Force Research Laboratory, AFRL/MLQL, 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida 32403-5323

The kinetics of trichloroethene (TCE) reductive dechlorination mediated by humic-Ni complexes in homogeneous aqueous solution using titanium(III) citrate as the bulk reductant was examined under various environmental conditions (e.g., pH and ionic compositions). Using Ca^{2+}, Zn^{2+}, and Hg^{2+} ions to vary Aldrich humic acid (HA)-Ni complex concentrations, pseudo-first-order rate constants for TCE reduction were observed to be proportional to HA-Ni levels (as calculated by speciation modeling), confirming HA-Ni complexes as the probable active mediator species. TCE reduction by HA-Ni was observed to be strongly pH dependent and could be due to both the variations of HA-Ni concentration and E_h with pH. Evidence is presented which suggests that quinone moieties may not be crucial for the humic-Ni mediated reduction of TCE. A variety of natural soil and aqueous humic material and Ni systems were examined, and some showed reactivity toward TCE. Humic-metal complexes may be important electron-transfer mediators in natural systems.

Introduction

Chlorinated organic solvents, such as trichloroethene (TCE), are common groundwater contaminants. Remediation of subsurface environments contaminated with chlorinated solvents has proven to be a major challenge. Reductive dechlorination reactions can affect the fate and bioavailability of chlorinated organic compounds in aqueous and subsurface environments. Some of these reactions may lead to incomplete dechlorination resulting in the formation of daughter products of similar or even greater toxicity than the parent compounds. TCE, for example, can be reduced to vinyl chloride, a known carcinogen. Recently, the complete reductive dechlorination of TCE to ethene and ethane mediated by humic-transition metal complexes has been reported by O'Loughlin et al. (*1*). The results of that study suggest that humic-transition metal complexes may represent a class of electron mediators in natural environments.

Suboxic environments are often sufficiently reducing to make reductive dechlorination reactions thermodynamically favorable, but the transfer of electrons from reduced species to a chlorinated solvent is often kinetically constrained. In the presence of reagents that can act as e^- transfer mediators, such as bacterial transition-metal coenzymes (e.g., vitamin B_{12} (Co) and F_{430} (Ni), and hematin (Fe)), the reaction rates can be greatly enhanced (*2−8*). O'Loughlin et al. (*1*) demonstrated that Aldrich humic acid (HA)-Ni and HA-Cu complexes, unlike the coenzymes previously examined, led to a greater extent of dechlorination for TCE. For instance, under similar experimental conditions, *cis*-1,2-dichloroethylene (*cis*-DCE) was the predominant product formed with vitamin B_{12}, coenzyme F_{430}, and hematin in the reductive dechlorination of TCE (*7*), while ethene and ethane were the major products with the humic-metal complex mediated reactions (*1*).

Humic substances (HSs) are heterogeneous polyfunctional polymers formed through the breakdown of plant and animal tissues by chemical and biological processes. HSs are probably the most widely distributed natural products on the earth's surface (*9*) and constitute from 10 to 30% of dissolved organic carbon (DOC) in groundwater to 70−90% in wetland waters (*10*). Although HSs often control many reactions occurring in soils and waters, their molecular configurations are usually not specifically determined (*9, 11−13*), which makes mechanistic studies of humic material-based reactions difficult. Natural organic matter (NOM) has recently been shown to be an effective mediator for the reduction of nitroaromatic compounds and polyhalogenated hydrocarbons in aqueous solution containing a bulk reductant (*14−16*). HSs are important components of NOM. Dunnivant et al. (*14*) proposed that in such systems possible mediators include quinone-type compounds and a variety of transition metal complexes, which are well-known to exist as constituents of NOM (*10, 17−19*). O'Loughlin and Burris (*16*) observed that Ni and Cu complexes with NOM in wetland sediment porewater and surface water were able to mediate the reductive dehalogenation of TCE using Ti(III) citrate as the bulk reductant. Some researchers believe that hydroquinone/quinone-type couple dominates the redox properties of HSs (*20*). Studies have shown that some quinone-type compounds can be efficient e^- transfer mediators for polyhalogenated alkanes with hydrogen sulfide as the bulk electron donor (*21−23*).

In this paper, we report on investigations into the effects of some environmental factors (pH, redox potential, competing cations, different sources, and fractions of HSs including modification of quinone moieties) on the reductive dechlorination of TCE mediated by humic-Ni complexes in homogeneous aqueous solution using titanium(III) citrate as the bulk electron donor. In the earlier study, humic-Ni complexes were found to be the most reactive of those examined (*1*). The goal of this study was to gain insights into the catalytic function of humic-Ni complexes on the reductive dechlorination of TCE. Although the experimental system used has an artificial character (use of Ti[III] as the bulk reductant and higher Ni concentrations compared to natural systems), it is used as a model system to assist in our understanding of the role of HSs in the fate of chlorinated ethenes in natural aquatic and subsurface environments. In addition, these reactions in engineered systems may lead to remedial alternatives for chlorinated solvent contamination.

Experimental Section

Chemicals and Materials. TCE was purchased from Fisher Scientific (Pittsburgh, PA). *cis*-DCE, *trans*-1,2-dichloroethylene (*trans*-DCE), 1,1-dichloroethylene (1,1-DCE), 1-butene, 2-butene (mixture of cis and trans), humic acid (sodium salt), sodium citrate (citric acid, trisodium salt), nickel chloride, poly(acrylic acid) (sodium salt), 2-hydroxy-1,4-naphthoquinone (lawsone), and 5-hydroxy-1,4-naphthoquinone (juglone) were from Aldrich (Milwaukee, WI). Vinyl chloride

* Corresponding author phone: (850)283-6035; fax: (850)283-6090; e-mail: david.burris@tyndall.af.mil.

(VC) in nitrogen, propane, propene, butane, and a gas mixture containing methane, ethane, ethylene, acetylene, carbon dioxide, and carbon monoxide were obtained from Alltech Associates (Deerfield, IL). n-Pentane was supplied by Burdick & Jackson (Muskegon, MI). Titanium(III) trichloride (13% in 20% HCl) was purchased from Fluka (Milwaukee, WI). 2-[N-morpholino]ethanesulfonic acid monohydrate (MES), N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), N-tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), sodium hydroxide, Trizma base, hydroquinone (99+%), quinalic acid, and Amberlite-CG 120 I cation-exchange resin (sodium form, polystyrene with sulfonic acid groups, 100−200 mesh, 4.4 equiv/kg total exchange capacity) were purchased from Sigma (St. Louis, MO). Zinc sulfate, mercuric chloride, calcium chloride, n-heptane, hydroxylamine hydrochloride, and 8-hydroxyquinoline were obtained from J. T. Baker (Phillipsburg, NJ).

Aldrich HA was extensively treated to remove the ash content, heavy metals, and residual fulvic acids (FA). Briefly, Aldrich HA was dissolved in 0.01 M NaOH followed by treatment with HF−HCl (24). Suwannee River FA and HA, Nordic FA and HA, peat HA, Summit Hill HA, Soil HA, and Leonardite HA were obtained from the International Humic Substances Society (IHHS); among these HSs only Suwannee River HA is standard grade, all others are reference grade. The collection and purification of Aberdeen Proving Ground DOC, Collier Mills Wildlife Management Area DOC, Tyndall AFB DOC, Dana HA, Charlisle HA, Rossmoyne HA, and Drummond Lake HA, FA and hydrophilic acid (HyI) were described elsewhere (16, 25−28). The HSs (except Drummond Lake HA, FA, and HyI) were sodium saturated using Na−Chelex 100 (Bio-Rad Laboratories, Hercules, CA) to remove trace metals. Excess sodium was removed by dialyzing against Milli-Q water using Spectra/Por CE 1000 MWCO cellulose ester dialysis tubing (Fisher Scientific, Pittsburgh, PA). Milli-Q (Millipore, Bedford, MA) distilled deionized water, argon-sparged for at least 1.5 h, was used to prepare all solutions in this study.

Experimental Reaction Systems and Procedures. All preparative work was performed in an anaerobic glovebox (Coy Laboratory Products, Ann Arbor, MI) with an atmosphere of 4−6% H_2 in N_2. Ti(III) citrate solution (250 mM in 760 mM Tris buffer, pH 8.0) was freshly prepared in the glovebox using a modified version (16) of the method described by Smith and Woods (29). The batch systems (160-mL serum bottles, crimp-sealed with Teflon-lined rubber septa, with final volumes of 100 mL of aqueous solution and 60 mL of headspace) were prepared, in duplicate, in the anaerobic chamber. More detailed procedures are provided in O'Louglin et al. (1). Typically, initial solution conditions were as follows: 5 or 10 mg OC/L of organic matter, 100 μM Ni^{2+}, 106 mM Tris buffer, and 15 mM Ti(III) citrate. Vials were spiked with 5 μL of a 1.0 M methanolic solution of n-heptane (or n-pentane) as the internal standard. The reaction was initiated by spiking with 2−8 μL of a 1.0 M methanolic solution of TCE. The bottles were placed on a roller drum and rotated vertically as the bottle axis remained horizontal and maintained at 20 °C in the dark.

For the pH effect experiments, the pH was controlled by sulfonic acid-based organic buffers (MES, for pH 5.5 to 6.5; HEPES, for pH 6.9 to 7.9; TAPS, for pH 7.9 to 9.2). These buffers were selected because of their low complexation of metals (30). No data were available regarding Ni complexation with these buffers. Typical experimental solutions in serum bottles contained 90 mL of 5.56 mg OC/L of Aldrich HA in 0.11 M buffer, 1 mL of 10^{-2} M Ni^{2+} (in 0.1 M HCl), and 0.1 mL of 1 M NaOH. The mixture was allowed to stay overnight in the glovebox. Then 6 mL of freshly prepared 250 mM Ti-(III) citrate was added. The solution pH was adjusted to 5.5−9.2 depending on different buffers presented and the target

pH value. The volume of liquid phase was adjusted to 100 mL with Milli-Q DI water. Redox potential (E_h) of the system was measured with a platinum redox electrode (Model 96-78, Orion, Boston, MA). The bottles were sealed and placed on a roller drum rotating vertically at 20 °C in the dark. After the kinetic experiment was started by adding TCE/n-heptane, headspace samples were analyzed at regular time intervals, depending on the expected reaction rates. Experiments were typically monitored through 2−3 reaction half-lives. After each experiment, pH and E_h were determined again.

For the experiments examining cation effects, 10^{-6} to 10^{-3} M of M^{2+} (Ca^{2+}, Zn^{2+} or Hg^{2+}) was equilibrated with HA-Ni complexes overnight (HA concentration was 5 mg OC/L, Ni^{2+} concentration was 2.5×10^{-5} M). It was found that at cation concentration as high as 10^{-3} M there was no visible precipitation observed in the humic solution. The preparation and addition of bulk reductant and the initiation of the experiment followed the procedures mentioned above.

Derivatization of Aldrich HA by Hydroxylamine. Oxygen-containing functionalities are responsible for many of the properties of HSs in the environment (10). Thorn et al. (31) showed from their ^{15}N NMR spectra that hydroxylamine reacted similarly with all FA and HA samples examined and could transform carbonyl functional groups including quinone functionalities to oximes. Hydroxylamine has been used in a number of studies to determine the carbonyl content of HSs (32−34). The reaction of hydroxylamine with carbonyl functional groups can be described simply as

$$R-\underset{\substack{\| \\ O}}{C}-R + H_2NOH \xrightarrow{-H_2O} R-\underset{\substack{\| \\ NOH}}{C}-R \qquad (1)$$
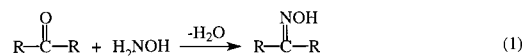
In this study, two doses of hydroxylamine were used to derivatize the Aldrich HA: one was similar to the dose used in the experiment of Thorn et al. (31), the other was overdosed to examine the effect of excess hydroxylamine on the TCE reduction rate. The derivatization of the Aldrich HA sample with hydroxylamine was performed by adding 0.1 mL of 10 mg/mL hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) (sample d-HA-1) or 100 mg of $NH_2OH \cdot HCl$ (overdosed, sample d-HA-2) into 300 mL of 11.11 mg/L HA solution. The mixture was titrated to pH 5 with 1 M NaOH and refluxed at 86−88 °C for 12 h. The derivatized HA samples were then used in the kinetic study of TCE reduction followed the procedures mentioned above.

Analytical Methods. Calibration standards containing TCE, DCEs (1,1-DCE, cis-DCE and trans-DCE), VC, and hydrocarbon gas components were prepared in 160-mL serum bottles with the same ratio of aqueous phase to vapor phase as the experimental systems (100 mL of water and 60 mL of headspace). For the consideration of air−water partitioning in the experimental system refer to reference (2). n-Heptane or n-pentane was used as the internal standard. Since the calibration system contained the same ratio of aqueous phase to vapor phase as the experimental systems, it accounted for water-vapor partitioning and allowed analyte concentrations in headspace samples to be expressed on a mass per vial basis. After equilibration of the mixture for 20 min (sufficient to reach equilibrium between vapor and liquid (2)), 100 μL headspace samples were analyzed using a Hewlett-Packard 5890 II GC with a GSQ PLOT column (0.53 mm × 30 m) and a flame ionization detector (FID). Samples were injected in split mode at 200 °C. Carrier gas was He at 4.6 mL/min and split vent at 9 mL/min. The FID temperature was 280 °C. The oven temperature program for a 20-min run (n-heptane as the internal standard) was 50 °C for 2 min, ramp 25 °C/min to 200 °C, and hold for 12 min. For reactions with fast kinetics, the column temperature was isothermal at 230 °C for a total

run time of 6 min, *n*-pentane was used as the internal standard, and only TCE was calibrated and quantitated.

WHAM Speciation Model. The Windermere Humic Aqueous Model (WHAM) is designed to calculate equilibrium chemical speciation in surface and groundwaters, sediments, and soils containing humic materials (*35*). The model is a combination of several submodels, which includes humic ion-binding Model V (*36–38*) and models of inorganic solution chemistry, etc. In Model V the binding of metal ions to HSs is described by a discrete binding sites model in which binding is modified by electrostatic interactions. Humic compounds carry proton-dissociating groups that can bind metal ions either singly or as bidentate pairs. Two types of proton-binding sites of the HSs are distinguished (denoted by A and B). Within each type there are four different groups, present in equal amounts, the p*K* values of which are described in terms of a median value, p*K*$_A$ or p*K*$_B$, and a factor, Δp*K*$_A$ or Δp*K*$_B$, that defines the range of the values. For example, the four type A groups have p*K* values given by

$$pK(i) = pK_A + (2i - 5)/6 \; \Delta pK_A \qquad (2)$$

where i = 1, 2, 3, or 4. For metal binding, the two types of site (A and B) have separate intrinsic exchange constants (p*K*$_{MHA}$ and p*K*$_{MHB}$). It is implicit in Model V that the variation in the binding affinities of the proton and metal ions on monodentate sites are correlated (i.e., a high proton affinity site also has a high affinity for all metal ions). Types A and B sites are respectively associated with carboxylic and phenolic type groups. The humic content of type B sites is fixed at one-half the content of the type A sites in the model.

In this study, one of two versions of WHAM, WHAM-W (for waters), was used to calculate the Ni speciation in various aqueous systems. The original database of the model was modified to include Ni-citrate, Ni-tris, and Ni–NH$_2$OH species. The default parameter values for FA and HA in the model were not changed. The WHAM model was used, primarily, to predict relative trends in concentrations.

Results and Discussion

The kinetics of reduction reactions examined in this study were pseudo-first-order with respect to TCE concentration. Therefore, the rate of disappearance of TCE can be described by

$$rate = - \frac{d[TCE]}{dt} = k_{obs}[TCE] \qquad (3)$$

and thus

$$[TCE]_t = [TCE]_0 e^{-k_{obs}t} \qquad (4)$$

where k_{obs} is the observed pseudo-first-order rate constant under given conditions and $[TCE]_t$ and $[TCE]_0$ are the TCE concentrations at time t and time zero, respectively. Representative rate plots are shown in Figure 1 for a given total Ni concentration of 10^{-4} M. The reduction of TCE was examined in two systems, each containing Ti(III) citrate as the bulk reductant: one with only Ni(II) (i.e., Ni system) and the other containing Ni–Aldrich HA complexes (i.e., Ni–HA system). The k_{obs} values were 1.12 h^{-1} and 0.14 h^{-1}, respectively, for systems with and without 5 mg OC/L of HA. The HA system was substantially faster than the system with Ni only.

Observed pseudo-first-order rate constants for the reduction of TCE as a function of Ni concentration for experimental systems with and without Aldrich HA present are shown in Figure 2. The kinetic behavior for both systems was nonlinear with respect to Ni concentration implying that there may be a limited number of reactive Ni sites in both systems. The
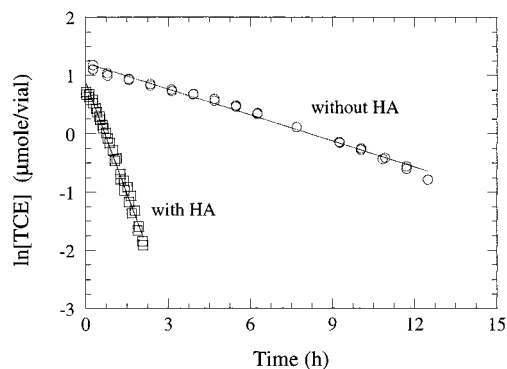

FIGURE 1. Representative plots of the disappearance of TCE from solutions containing 0.1 mM Ni, 15 mM Ti(III) citrate, and 106 mM pH 8.0 Tris buffer with and without 5 mg OC/L of Aldrich HA. The lines are linear fitting results. (Plots are of data from duplicate vials.)
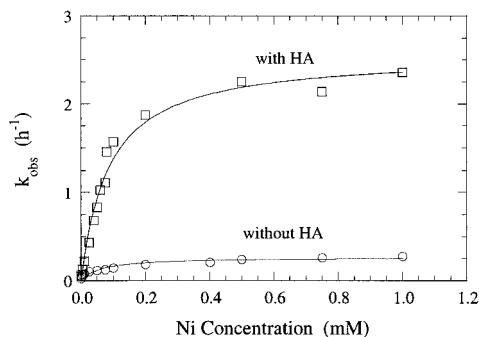

FIGURE 2. Observed pseudo-first-order rate constant, k_{obs}, as a function of total Ni concentration in systems with and without HA present. Experimental conditions: 15 mM Ti(III) citrate, 106 mM pH 8.0 Tris buffer, 0–1 mM Ni, and 0 or 5 mg OC/L of Aldrich HA. The lines are the best fit of a Langmuir type expression to the experimental results and are shown for visualization purposes.

maximum rate of TCE reduction in the system with HA was almost 10-fold higher than the system without HA, indicating that HA is essential for the much faster reaction. O'Loughlin et al. (*1*) showed that while Ti(III) citrate was a strong reductant, in the absence of an effective e^- mediator, it was essentially nonreactive with an respect to the reductive dechlorination of TCE. In addition, although humic materials have been shown to be effective e^- transfer mediators in the reduction of nitroaromatic compounds, carbon tetrachloride, and hexachloroethane (*14, 15*), the Aldrich HA alone (i.e., without the addition of Ni) was not an effective e^- mediator for the reduction of TCE by Ti(III) citrate (*1*).

To understand relative reactivities of various species in these systems, the speciation of Ni was calculated with the WHAM model. The results of speciation calculation by WHAM for the Ni and Ni–HA systems are listed in Table 1. Within the range of Ni concentrations examined in this study (10^{-6}–10^{-3} M), the percentage of each species was the same, so the results of Ni species in Table 1 were expressed as the percentage of total Ni concentration. The calculation results revealed that both systems contain nearly identical Ni species and also identical concentrations of various Ni species at a given total Ni concentration (except, of course, for Ni–HA complexes in the system containing HA). Ni(citrate), Ni(Tris)$_2$, and Ni(Tris) were major species and consisted of 96.4%, 3.24%, and 0.385% of total Ni concentration, respectively. Figures 1 and 2 clearly indicate that the reduction rate of TCE in the system containing Ni and HA is significantly greater than that in the system containing only Ni at a given total Ni concentration. This difference can be hypothesized as being due to the presence of Ni–HA complexes in the

TABLE 1. Speciation Calculations by the WHAM Model for Systems Containing Ni with and without HA[a,b]

species	% total Ni concentration	
	Ni system	Ni—HA system
Ni^{2+}	9.14×10^{-3}	9.14×10^{-3}
$NiOH^+$	6.15×10^{-5}	6.15×10^{-5}
$Ni(OH)_2$ aq	3.15×10^{-6}	3.15×10^{-6}
Ni(Tris)	0.385	0.385
$Ni(Tris)_2$	3.24	3.24
Ni(Citrate)	96.4	96.4
Ni(Citrate)H	2.04×10^{-9}	2.04×10^{-9}
$Ni(Citrate)H_2$	3.12×10^{-19}	3.12×10^{-19}
Ni—HA		4.94×10^{-3}
total	100	100

[a] Experimental conditions: 15 mM Ti(III) citrate ([citrate] = 0.035 M), 106 mM pH 8.0 Tris buffer, 0.001–1 mM Ni, and 0 or 5 mg OC/L of Aldrich HA. [b] Ti(III) species were not included in the speciation calculations due to the unavailability of necessary speciation constants. Absence of Ti speciation should not effect relative distributions of Ni species present in the table.
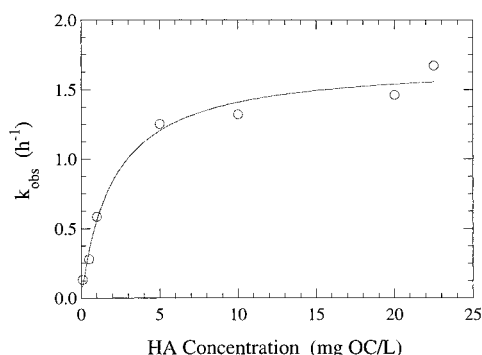

FIGURE 3. Observed pseudo-first-order rate constant, k_{obs}, as a function of total HA concentration in the Ni—HA system. Experimental conditions similar to those given in Figure 2, Ni concentration was fixed at 100 μM. The line is the best fit of a Langmuir type expression to the experimental results and is shown for visualization purposes.

system with HA, though they only account for less than 0.01% of total Ni concentration. The results of these experiments and speciation calculations support the hypothesis that Ni—HA complexes are responsible for the faster reduction reaction (i.e., they are effective e^- transfer mediators). Note that Table 1 also illustrates a limitation of WHAM in that it predicts a linear relationship between HA-Ni complexes and total Ni, where as it is indicated to be nonlinear in Figure 2 (i.e., WHAM, as it was used here, should be useful for lower metal concentrations where HA-metal concentrations are in the linear region.)

The relationship between k_{obs} values for the TCE reduction reaction and HA concentration in the Ni—HA system is shown in Figure 3. In this experiment, only HA concentration was changed; all other conditions, including total Ni concentration, were fixed. The speciation calculation results (not shown) indicated that Ni—HA concentration increased with increasing HA concentration, while concentrations of all other Ni species remained nearly constant. The result also supports the conclusion derived above.

Three metal ions (Ca^{2+}, Zn^{2+}, and Hg^{2+}) were selected to assess the influence of competitive binding on the reductive dechlorination of TCE in the Ni—HA system according to the well-known Irving-Williams order of stability constants of humic complexes for various metals (39):
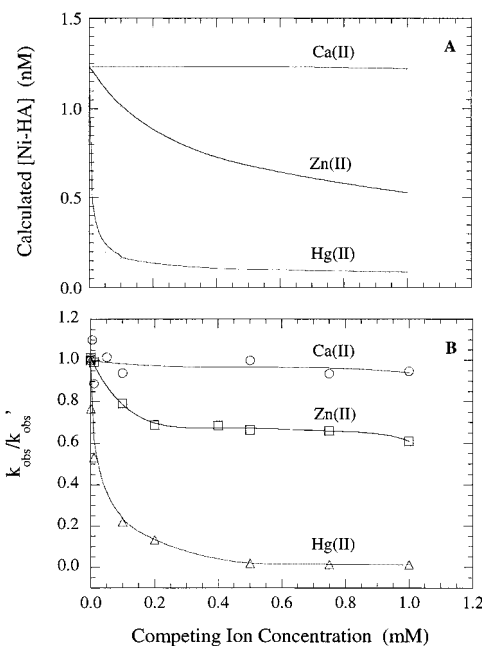
$$Mg(II) < Ca(II) < Cd(II) \approx Mn(II) < Co(II) < Zn(II) \approx$$
$$Ni(II) < Cu(II) < Hg(II)$$

FIGURE 4. (A) Estimated Ni—HA concentrations for the Ni—HA system in the presence of competing ions (Ca^{2+}, Zn^{2+}, and Hg^{2+}) using the WHAM speciation model (see text for modeling details). (B) Observed pseudo-first-order rate constants for the reduction of TCE in the Ni—HA system in the presence of competing ions (Ca^{2+}, Zn^{2+}, and Hg^{2+}) normalized to K'_{obs}, the k_{obs} without the competing ion present.

Ca(II) is ubiquitous in natural waters and has a low affinity for humic materials. Hg(II) can form the strongest complexes with humics, while Zn(II) and Ni(II) have similar affinities for humics. It was hypothesized that these cations affect the reduction rate of TCE by competing for available HA binding sites, thus reducing the concentration of Ni—HA complex. Ti(III) conditions were the same throughout the experiments, so the effect of potential Ti(III) binding to HA sites on the relative impact of Ca(II), Zn(II), and Hg(II) competing for sites occupied by Ni should be minimal. The WHAM model was used to examine the cation effects on Ni—HA concentration because the model considers both competition among cations for the binding sites on HA and electrostatic effects. The results of speciation calculations expressed as Ni—HA concentration versus competing ion concentration are shown in Figure 4A.

The TCE reduction kinetic results in the Ni—HA system as a function of competing ion (Ca^{2+}, Zn^{2+}, and Hg^{2+}) concentration are given in Figure 4B as rate constants normalized to K'_{obs}, the pseudo-first-order rate constant in the Ni—HA system without the competing ion present (i.e., k_{obs}/K'_{obs}). O'Loughlin et al. (1) reported that the Zn—HA complex did not demonstrate activity as an e^- transfer mediator in the reductive dechlorination of TCE; similarly, in this study the Ca—HA and Hg—HA complexes were not observed to mediate the reductive dechlorination of TCE (data not shown). The kinetic results shown in Figure 4B have a pattern similar to that for the Ni—HA concentrations estimated in Figure 4A. The addition of Ca^{2+} had little, if any, effect on reduction rates of TCE since Ca^{2+} addition barely lowered the Ni—HA concentration. Zn^{2+} and especially Hg^{2+} additions had a much greater effect on TCE reaction rates since they competed effectively for the same binding sites required by Ni^{2+}. These experimental and modeling results provide further confirmation that Ni—HA complexes are the likely e^- transport mediation sites for the reductive dechlorination of TCE in the Ni—HA system.
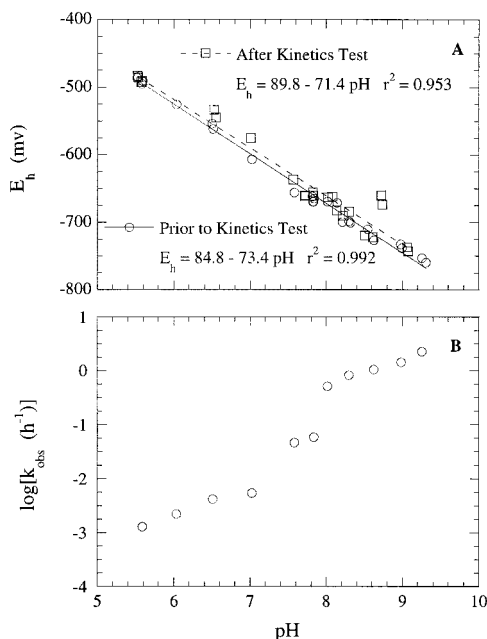
FIGURE 5. (A) Redox potentials of solutions in the Ni–HA system (see Experimental Section for experimental details) before and after the reduction of TCE as a function of pH. (B) Observed pseudo-first-order rate constants (logarithm form) of TCE reduction in the Ni–HA system as a function of pH. log–log plot used for clarity of data presentation.
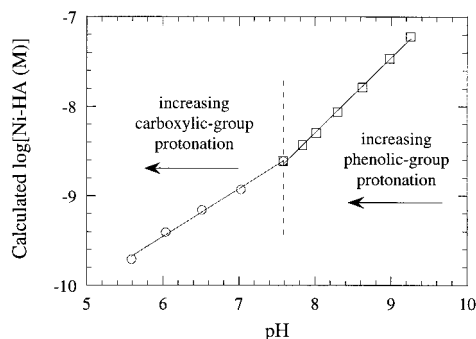

FIGURE 6. Ni–HA concentrations (in logarithm form) calculated by the WHAM model as a function of pH for experimental system shown in Figure 5. Regions of increasing protonation of phenolic- and carboxylic-groups are indicated.

The effect of pH on the TCE transformation rate was studied over the pH range of 5.5–9.2 at constant Ni and HA concentrations. In Figure 5, the relationship between pH and E_h (Figure 5A) and the observed k_{obs} values as a function of pH (Figure 5B) are shown. Increasing solution pH caused a significant increase in the rate of reductive dechlorination of TCE, increasing more than 3 orders of magnitude from pH 5.5 to 9.2.

To evaluate the pH dependence of k_{obs} several factors have to be considered. First, in the Ni–HA system the E_h of the solution changed with pH. E_h linearly decreased with increasing pH (Figure 5A), which was consistent with the results of Zehnder (40). He measured the redox potential of a 2 mM solution of Ti(III)/Ti(IV) in 0.02 M citric acid as a function of pH and observed a linear relationship between pH and E_h over a pH range of 5–10. Thus, the higher the pH, the lower the reduction potential of the system, which should favor TCE reduction. Some researchers proposed that the reduction potentials of many of the reactive NOM components could exhibit strong pH dependence (14, 22). The increase in k_{obs} with decreasing E_h may be due, in part, to the reduction of additional reactive NOM components exhibiting a more negative reduction potential (14). Second, pH had a dramatic effect on the extent of metal binding by humic materials as for any weak acid ligand whose apparent affinity for a metal increases with pH. Changing pH affects metal-humic formation via competition between proton and metal ion for binding sites on HSs, competition between HSs and other ligands for the metal, and electrostatic and conformational effects of pH on the ligand (41). An increasing number of reactive sites (e.g., the phenolate species) in HSs would be created at increasing pH due to the deprotonation of phenolic groups (14). These mixed effects result in increased metal binding at increasing pH (42), thus TCE reduction rates should increase. It should be noted that if there was significant binding of Ni to the buffers used (TAPS, HEPES, and MES), it would been reflected in decreasing k_{obs} with increasing pH for a given buffer. As seen in Figure 5B, these trends were not observed. It is interesting to note that

the data in Figure 5B has the appearance of a titration curve. Figure 6 shows the calculated Ni–HA concentrations by the WHAM model vs pH. Ni–HA concentrations increase with pH in two separate regions, the lower pH region corresponding to the carboxyl binding sites and the upper region corresponds to the phenolate sites (the directions of increasing protonation of the binding sites are indicated) (10). These two regions appear to be represented in the titration curve behavior in Figure 5B with the shift between the dominant binding sites occurring in the pH 7–8 region. This further illustrates that increasing pH increases Ni–HA concentration and therefore can lead to increased rates of TCE transformation. Thus, the pH effect on the TCE reduction rate may be due to the variations of both Ni–HA concentration and E_h with pH.

Some studies (14, 15) have shown that quinones (including model compounds and naturally occurring quinones) could mediate the reduction of polyhalogenated alkanes and nitroaromatic compounds in the presence of hydrogen sulfide as the bulk reductant. The findings of Perlinger et al. (21, 23) suggest that mercapto-quinone groups may be redox-active sites in HSs. To determine if quinones are effective e^- mediators in the reductive dechlorination of TCE using Ti-(III) citrate as the bulk reductant, several model quinone compounds as well as naturally occurring quinones were examined in this study. The experimental conditions were similar to those of HA experiments, except that the solution contained 10 mg OC/L of each quinone compound instead of HA. Table 2 lists the k_{obs} values of TCE reduction reaction for these quinone compounds and two carboxylic compounds with and without Ni, and for comparison the k_{obs} values for the Aldrich HA and the control without HA or organic compounds. It is clear from Table 2 that neither the complexes of Ni with these quinones or carboxylic compounds nor the compounds alone are effective e^- mediators in the reductive dechlorination of TCE by Ti(III) citrate. It is noted that for quinone compounds (except lawsone) the compounds alone are a little more active in TCE reduction than the corresponding Ni complexes. Furthermore, unlike hydrogen sulfide that was shown to react with juglone and the products had given reactivities with respect to hexachloroethane transformation (21), our results did not show the evidence that Ti(III) citrate could change the activities of quinone groups as e^- transfer mediators for the reduction of TCE.

Two additional experiments were performed to further examine the possible activity of quinone groups in natural humic materials as mediators in the reductive dehalogenation of TCE. In the first experiment, three fractions (HA, FA, and HyI) of NOM collected from Drummond Lake in the Great Dismal Swamp in Virginia (28) were used to examine TCE reduction following the same procedures for the Aldrich HA and quinone experiments. Table 3 presents the k_{obs} values

TABLE 2. Pseudo-First-Order Rate Constants of TCE Reduction in the Presence of Model Quinone Groups, Carboxylic Groups, and Aldrich HA[a]

Name	Structure	k_{obs} (h^{-1})[b]	
		With Ni	Without Ni
8-Hydroxyquinoline		0.087 ± 0.003	0.16 ± 0.01
Hydroquinone		0.077 ± 0.003	0.095 ± 0.004
Juglone		0.098 ± 0.006	0.10 ± 0.01
Lawsone		0.087 ± 0.006	0.064 ± 0.004
Quinalic Acid		0.069 ± 0.002	0.053 ± 0.002
Polyacrylic acid		0.025 ± 0.001	0.025 ± 0.002
Aldrich Humic Acid	—	1.32 ± 0.07	0.052 ± 0.001
Control (DI H$_2$O)	—	0.14 ± 0.01	0.029 ± 0.003

[a] Experimental conditions: 10 mg OC/L organic matter with or without 0.1 mM Ni, 15 mM Ti(III) citrate, 106 mM Tris buffer, and pH = 8.0. [b] Uncertainties represent 95% confidence limits.

TABLE 3. Pseudo-First-Order Rate Constants of TCE Reduction for Three Fractions (FA, HA, and HyI) of Drummond Lake NOM at Two pH Values and for Samples d-HA-1 and d-HA-2[a] and Aldrich HA[b]

DOC	pH	k_{obs}[c] (h^{-1})
Drummond Lake FA	7.9	0.38 ± 0.02
Drummond Lake HA	7.9	0.37 ± 0.02
Drummond Lake HyI	7.9	0.35 ± 0.02
Drummond Lake FA	7.2	0.064 ± 0.004
Drummond Lake HA	7.2	0.059 ± 0.002
Drummond Lake HyI	7.2	0.074 ± 0.003
Aldrich HA	8.0	1.32 ± 0.07
Sample d-HA-1	8.0	1.22 ± 0.05
Sample d-HA-2	8.0	0.058 ± 0.002

[a] Hydroxylamine derivatized Aldrich HA samples, see text for details. [b] Experimental conditions: 10 mg OC/L organic matter, 0.1 mM Ni, 15 mM Ti(III) citrate, 106 mM Tris buffer, and pH values as shown. [c] Uncertainties represent 95% confidence limits.

for the three fractions at two pH values. At each pH, k_{obs} of the HyI fraction was quite similar to those of FA and HA fractions. From structural characterization analyses, it was proposed that HyI fractions isolated from NOM samples were nonhumic materials (28). Furthermore, ¹H NMR spectra (28) revealed that no peak was found at chemical shifts above 6 ppm (6.0−8.5 ppm range is generally attributed to aromatic protons including quinones, phenols, and oxygen containing heteroaromatics) in the HyI fraction, indicating that the HyI fraction was devoid of aromatic protons. This result suggested two possibilities. First, quinones in HSs may not be important in the mediation of TCE reduction reaction. If quinones were the main functional groups responsible for the mediation of TCE reduction, a significant difference should be observed between the rate constants for the HyI fraction and HA (or FA) fraction. Second, since the HyI fraction consisted of more simple compounds that were nonhumic nature, and nearly no difference was observed among k_{obs} values for the three fractions, it would be possible that complexes of Ni with

TABLE 4. Pseudo-First-Order Rate Constants for TCE Reduction in the Presence of Different Sources of DOC[a]

source of DOC	origin[b]	k_{obs}[c] (h^{-1})
Aberdeen Proving Ground DOC[d]	aq	0.87 ± 0.04
Tyndall AFB DOC[e]	aq	0.82 ± 0.10
Charlisle HA	T-soil	0.56 ± 0.03
Leonardite HA	T-coal	0.40 ± 0.01
Drummond Lake HA	aq	0.38 ± 0.02
Drummond Lake FA	aq	0.37 ± 0.02
Drummond Lake HyI	aq	0.35 ± 0.02
Collier Mills Wildlife Management Area DOC[f]	aq	0.33 ± 0.01
Nordic HA	aq	0.26 ± 0.01
Nordic FA	aq	0.21 ± 0.01
Suwannee River HA	aq	0.19 ± 0.01
Suwannee River FA	aq	0.18 ± 0.01
Peat HA	T-soil	0.093 ± 0.002
Dana HA	T-soil	0.090 ± 0.002
Soil HA	T-soil	0.088 ± 0.002
Summit Hill HA	T-soil	0.078 ± 0.003
Rossmoyne HA	T-soil	0.066 ± 0.002
control (DI H$_2$O)		0.14 ± 0.01
Aldrich HA	T-coal	1.12 ± 0.05

[a] Experimental conditions: 5 mg OC/L organic matter, 0.1 mM Ni, 15 mM Ti(III) citrate, 106 mM Tris buffer, and pH = 8.0. [b] Origin-aq: aquatic OC; T-coal: territorial coal type OC; and T-soil: territorial soil type OC. [c] Uncertainties represent 95% confidence limits. [d] DOC = 11.5 mg OC/L (see ref 16). [e] DOC = 30.7 mg OC/L (see ref 16). [f] DOC = 13.4 mg OC/L (see ref 16).

simple organic matter, not necessarily HSs, could also act as effective e^- transfer mediators in the reductive dechlorination of TCE. More studies are needed to investigate active groups or structures in HSs that are responsible for e^- transfer.

In the second experiment, complexes of Ni with hydroxylamine-derivatized Aldrich HA (samples d-HA-1 and d-HA-2) were tested for their activities as e^- mediators in the reductive dechlorination of TCE. As indicated in the Experimental Section, the concentration ratio of hydroxylamine to HA in sample d-HA-1 was similar to that used in the study of Thorn et al. (31), while in sample d-HA-2 the ratio was 100-times higher than that of sample d-HA-1. Thorn et al. (31) provided strong confirmatory evidence for the presence of quinones in humic and fulvic acids of diverse origins and the derivatization of carbonyl groups (including quinones) into oximes. Table 3 shows the k_{obs} values of TCE disappearance for two hydroxylamine-derivatized HA samples compared with the Aldrich HA. The sample d-HA-1 had a similar k_{obs} value to that of underivatized HA, suggesting that quinones may not be responsible for the e^- transfer activity of Ni−HA complexes. For the sample d-HA-2, however, the derivatized HA was totally unreactive compared to the underivatized HA. It is not clear what kinds of reactions are undergoing with excessive hydroxylamine. The speciation calculation (results not shown) indicates that Ni−NH$_2$OH complexes account for 50.5% of total Ni concentration in the d-HA-2 system. In contrast, Ni-citrate complexes only consist of 47.7%, compared to 96.4% in the system containing underivatized HA. It is not clear what the mixed results of the derivatized HA experiments indicate.

The results of this study and O'Loughlin et al. (1) demonstrate that complexes of Ni with Aldrich HA can effectively mediate the reduction of TCE using Ti(III) citrate as the bulk reductant. O'Loughlin and Burris (16) also observed that Ni and Cu complexes with naturally occurring dissolved organic matter collected from three wetland samples were able to mediate the reductive dehalogenation of TCE (Table 4). TCE transformation mediated by these complexes was relatively complete (with final products of ethylene and ethane, almost devoid of other intermediates

TABLE 5. Stability Constants (K_i) and Binding Site Concentrations (C_i) from Copper Titrations (44) of Humic Substances and Pseudo-First-Order Rate Constants for TCE Reduction in the Presence of These NOM Samples[a]

		log K_i (L/mol)			C_i (mmol/g C)			
DOC[b]	k_{obs}[c] (h^{-1})	i[d] $= 1$	$i = 2$	$i = 3$	$i = 1$	$i = 2$	$i = 3$	r^2
Aldrich HA	1.12	11.40	8.97	7.20	0.17	0.49	1.79	0.999
Charlisle HA	0.56	11.64	8.91	7.16	0.26	0.59	2.25	0.998
Leonardite HA	0.40	10.90	8.85	7.13	0.25	0.56	1.64	0.998
Drummond Lake HA	0.38	11.07	8.76	7.26	0.15	0.49	1.52	0.999
Drummond Lake FA	0.37	11.46	9.13	7.62	0.12	0.38	1.19	1.000
Nordic HA	0.26	10.27	8.50	7.25	0.19	0.38	1.23	1.000
Nordic FA	0.21	10.66	8.63	7.22	0.17	0.36	1.29	1.000
Suwannee River HA	0.19	10.47	8.59	7.16	0.13	0.38	1.29	1.000
Suwannee River FA	0.18	10.33	8.49	7.01	0.10	0.40	1.36	1.000
Peat HA	0.093	11.87	9.51	8.10	0.26	0.36	1.01	0.999
Dana HA	0.090	12.47	10.14	8.39	0.29	0.21	1.19	0.998
Soil HA	0.088	11.48	9.25	7.93	0.43	0.45	1.10	0.995
Summit Hill HA	0.078	11.61	9.23	7.75	0.20	0.41	0.97	1.000
Rossmoyne HA	0.066	11.72	8.92	7.22	0.28	0.49	1.55	0.999

[a] The copper titration conditions: 5 mg OC/L organic matter in DI H_2O, and pH was adjusted to 7.20 using 0.1 N HNO_3 or NaOH. [b] For the origins of these samples see Table 4. [c] Only average values are reported. For the 95% confidence limits see Table 4. [d] The number of site

during the reaction or those compounds under detection limit) and rapid (with half-lives on the order of minutes to hours under our experimental conditions). To extrapolate our results to the mediation activity of Ni complexes with NOM including humic materials (humic and fulvic acids) of diverse origins, additional natural DOC and HSs samples were collected and examined. Table 4 presents the pseudo-first-order rate constants for TCE reduction in the presence of different sources of DOC and HSs under the same experimental conditions used with the Aldrich HA. Some of these natural DOC and HSs from sources such as anaerobic wetland water, soil, river water, and groundwater are shown to have activity in the mediation of TCE transformation in laboratory systems, while some (mainly from soils) are not reactive. It should be noted that Ti(III) citrate is an exceptionally strong reductant and is not representative of the "bulk" reductants typically found in natural environments. Some environmentally relevant bulk reductants (such as green rust, FeS, and NaS) were examined in our laboratory systems, and none were found to reduce TCE in the presence of Ni− or Cu-DOC complexes. Further investigation of active and nonactive DOC and HSs in Table 4 is necessary and may lead to a better understanding of the components or even structures that are responsible for the faster reduction.

Efforts were made to relate the rate constants of TCE reduction to the extent of Ni complexation by the array of NOM examined. A resin equilibrium method (43) was used to study the complexation of Ni by NOM, but meaningful results were not obtained. So, copper titrations (44) of these NOM samples were performed based on the assumption that Cu and Ni react similarly with DOC. Table 5 shows the stability constants and binding site concentrations, along with k_{obs} values for TCE reduction of these NOM samples. No significant correlation was observed between k_{obs} values and fitted binding parameters, indicating that the nature of binding sites or functional groups in NOM is very important.

In this study, factors affecting the reductive dechlorination of TCE mediated by Ni-humic complexes using Ti(III) citrate as the bulk reductant were examined. TCE reduction k_{obs} values for a given HA concentration increase to a plateau with increasing total Ni concentration. Likewise, TCE reduction k_{obs} values for a given total Ni concentration increase to a plateau with increasing HA concentration. Zn^{2+} and Hg^{2+}, ions capable of competing for Ni-binding sites on HA, were found to lower TCE reduction k_{obs} values in proportion to their ability to displace Ni (as determined by speciation modeling). These results confirm that the Ni−HA complex acts as an effective electron transport mediator. Increasing

pH conditions were found to increase the TCE reduction k_{obs} values. The effect of pH on TCE k_{obs} could be due to several factors including the following: (1) E_h decreases with increasing pH in the Ti(III) citrate system and (2) increasing pH increases Ni−HA concentrations. Examination of the role of HA quinone groups suggests that quinone groups may not play a vital role in the reaction. Several natural dissolved organic materials and humic and fulvic acids also had the ability to accelerate the rate of TCE disappearance in the presence of Ni. The results of this investigation suggests that transition metal-humic complexes may represent a class of environmentally relevant e^- transfer mediators.

Literature Cited
(1) O'Loughlin, E. J.; Burris, D. R.; Delcomyn, C. A. *Environ. Sci. Technol.* **1999**, *33*, 1145−1147.
(2) Burris, D. R.; Delcomyn, C. A.; Smith, M. H.; Roberts, A. L. *Environ. Sci Technol.* **1996**, *30*, 3047−3052.
(3) Chiu, P.-C.; Reinhard, M. *Environ. Sci. Technol.* **1995**, *29*, 595−603.
(4) Krone, U. E.; Thauer, R. K.; Hogenkamp, H. P. C. *Biochemistry* **1989**, *28*, 4908−4914.
(5) Krone, U. E.; Thauer, R. K.; Hogenkamp, H. P. C. *Biochemistry* **1989**, *28*, 10061−10065.
(6) Klecka, G. M.; Gonsior, S. J. *Chemosphere* **1984**, *13*, 391−402.
(7) Gantzer, C. J.; Wackett, L. P. *Environ. Sci. Technol.* **1991**, *25*, 715−722.
(8) Glod, G.; Brodmann, U.; Angst, W.; Holloger, C.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1997**, *31*, 3154−3160.
(9) Schnitzer, M.; Khan, S. U. *Humic Substances in the Environment*; Marcel Dekker: New York, 1972.
(10) Thurman, E. M. *Organic Geochemistry of Natural Waters*; Martinus Nijhoff/Dr W. Junk Publishers: Boston, 1985.
(11) Schnitzer, M. *Humic Substances: Chemistry and Reactions*; Elsevier/North-Holland: Amsterdam, The Netherlands, 1978.
(12) Stevenson, F. J. *Humus Chemistry*; John Wiley & Sons: New York, 1982.
(13) Bogg, S. Jr.; Livermore, D.; Seltz, M. G. *Humic Substances in Natural Waters and Their Complexation with Trace Metals and Radionuclides: A Review*; ANL-84-78, Argonne National Laboratory: Argonne, Illinois, 1984.
(14) Dunnivant, F. M.; Schwarzenbach, R. P.; Macalady, D. L. *Environ. Sci. Technol.* **1992**, *26*, 2133−2141.
(15) Curtis, G. P.; Reinhard, M. *Environ. Sci. Technol.* **1994**, *28*, 2393−2401.

(16) O'Loughlin, E. J.; Burris, D. R. In *Wetlands and Remediation*; Means, J. L., Hinchee, R. E., Eds.; Battelle Press: Columbus, OH, 2000; pp 1−8.

(17) Stevenson, F. J.; Ardakani, M. S. In *Micronutrients in Agriculture*; Mortuedt, J. J., Giordano, P. M., Linsay, W. L., Eds.; Soil Science Society of America: Madison, WI, 1972.

(18) Hayes, M. H. B.; Swift, R. S. In *The Chemistry of Soil Constitutents*; Greenland, D. J., Hayes, M. H. B., Eds.; Wiley: New York, 1978.

(19) Waite, T. D. In *Humic Substances II. Interactions with Metals, Minerals, and Organic Chemicals*; MacCarthy, P., Hayes, M. H. B., Malcolm, R. L., Swift, R. S., Eds.; Wiley-Interscience: Chichester, England, 1990.

(20) Senesi, N.; Schnitzer, M. *Soil Sci.* **1977**, *23*, 224−234.

(21) Perlinger, J. A.; Angst, W.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1996**, *30*, 3408−3417.

(22) Schwarzenbach, R. P.; Stierli, R.; Lanz, K.; Zeyer, J. *Environ. Sci. Technol.* **1990**, *24*, 1566−1575.

(23) Perlinger, J. A.; Buschmann, J.; Angst, W.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1998**, *32*, 2431−2437.

(24) Schnitzer, M. In *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*; Page, A. L., Miller, R. H., Keeney, D. R., Eds.; American Society of Agronomy and Soil Science Society of America: Madison, WI, 1982; pp 581−594.

(25) Novak, J. M.; Smeck, N. E. *Soil Sci. Soc. Am. J.* **1991**, *55*, 96−102.

(26) Traina, S. J.; Spontak, D. A.; Logan, T. J. *J. Environ. Qual.* **1989**, *18*, 221−227.

(27) O'Loughlin, E. J.; Traina, S. J.; Chin, Y.-P. *Environ. Toxicol. Chem.* **2000**, *19*, 2015−2021.

(28) Ma, H.; Allen, H. E.; Yin, Y. *Water Res.*, in press.

(29) Smith, M. H.; Woods, S. L. *Appl. Environ. Microbiol.* **1994**, *60*, 4107−4110.

(30) Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. M. *Biochemistry* **1966**, *5*, 467−477.

(31) Thorn, K. A.; Arterburn, J. B.; Mikita, M. A. *Environ. Sci. Technol.* **1992**, *26*, 107−116.

(32) Schnitzer, M.; Gupta, U. C. *Soil Sci. Soc. Am. Proc.* **1964**, *28*, 374−377.

(33) Schnitzer, M.; Skinner, S. I. M. *Soil Sci.* **1966**, *101*, 120−124.

(34) Porter, L. K. *Soil Sci. Soc. Am. Proc.* **1969**, *33*, 696−702.

(35) Tipping, E. *Computers Geosciences* **1994**, *20*, 973−1023.

(36) Tipping, E.; Hurley, M. A. *Geochim. Cosmochim. Acta* **1992**, *56*, 3627−3641.

(37) Tipping, E. *Environ. Sci. Technol.* **1993**, *27*, 520−529.

(38) Tipping, E. *Coll. Surf.* **1993**, *73*, 117−131.

(39) Mantoura, R. F. C.; Dickson, A.; Riley, J. P. *Estuar. Coastal Mar. Sci.* **1978**, *6*, 386-408.

(40) Zehnder, A. J. B. *Thesis Swiss Federal Institute of Technology (ETH)*; Dissertation ETH 5716, 1976.

(41) Cabaniss, S. E.; Shuman, M. S. *Geochim. Cosmochim. Acta* **1988**, *52*, 185−193.

(42) Buffle, J. In *Metal Ions in Biological Systems*; Sigel, H., Eds.; Marcel Dekker: New York, 1984; pp 165−221.

(43) Christensen, J. B.; Christensen, T. H. *Environ. Sci. Technol.* **1999**, *33*, 3857−3863.

(44) Ma, H.; Kim, S. D.; Cha, D. K.; Allen, H. E. *Environ. Toxicol. Chem.* **1999**, *18*, 828−837.